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International diagnostic accuracy study for the serological detection of chikungunya virus infection

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Abstract

External quality assurance for serological detection of chikungunya virus infection was performed to assess the diagnostic quality of expert laboratories. Of 30 participants, only six correctly analysed all reference samples with their respective tests. Thirteen laboratories gave at least 85% correct results, and 11 laboratories 75% or less. IgM antibodies were detected less frequently than IgG antibodies ($p < 0.001$). The study provides information on the quality of different serological tests and indicates that most of the participants need to improve the sensitivity of their assays, in particular to detect IgM antibodies more reliably and be able to detect acute infections adequately.

Keywords: Chikungunya fever, emerging viral infection, external quality assurance, serology, virus diagnostic

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Chikungunya virus (CHIKV), an alphavirus of the family *Togaviridae*, received considerable attention during its last major outbreaks in the Indian Ocean region, India and Southeast Asia in 2005–2006. It is transmitted to humans by *Aedes* mosquitoes. The infection is characterized by sudden onset of fever, skin rash and severe arthralgia, sometimes persisting for several months. Currently, there is no effective antiviral treatment, but symptoms are generally self-limiting [1–5]. In summer 2007, the first autochthonous CHIKV outbreak in Europe was reported from north-eastern Italy [6]. As *Aedes albopictus* has been introduced into several European countries, fears exist that viral transmission could also resurge in other countries, because of the introduction of viraemic persons from endemic areas and ecological factors [7]. Reliable laboratory diagnostics are required for detection of imported cases, and early recognition of local transmission to strengthen surveillance and timely vector control. During CHIKV infections, IgM and IgG antibodies can be detected shortly after the onset of symptoms [8] both by in-house assays and by the few existing commercial assays [1,9]. However, the sensitivity and specificity of these tests have been poorly assessed, and the possibility of false-positive reactions resulting from cross-reactivity with other arboviruses should be excluded.

We present the results of the first external quality assurance (EQA) study on CHIKV serology to assess the diagnostic accuracy of laboratories and procedures. Thirty expert laboratories from 23 countries participated in this study, announced as EQA on diagnostic proficiency, run by the European Network for diagnostics of 'Imported' Viral Diseases, and including publication of the results in a comparative and anonymous manner. Test samples were generated by diluting well-characterized human sera with fresh frozen plasma negative for human immunodeficiency virus, hepatitis B virus and hepatitis C virus. After dilution, the enriched serum samples were heat-inactivated (56°C, 1 h), frozen, and lyophilized in aliquots of 100 μL. Proficiency panels consisted of ten reference samples. As positive controls, aliquots of six samples from three selected sera positive for antibodies against CHIKV (including a two-fold dilution series of one antiserum: samples 12, 11, 2 and 4) were provided. For specificity controls, aliquots of two antisera containing antibodies reactive with heterologous arboviruses (dengue virus and West Nile virus), approved in past EQA panels [10,11], were included. Two aliquots from confirmed seronegative samples served as negative controls. Before shipment, two EQA panel sets were tested by two reference laboratories in our network, to confirm the quality of the samples after preparation. Participants were asked to analyse the material by the diagnostic methods that they routinely use for serological detection of CHIKV. Information was requested concerning the method type/format, e.g. enzyme immunoassay, haemagglutination inhibition assay or immunofluorescence assay, and whether it was an in-house assay or commercial kit. The following criteria were selected as minimum requirements for successful overall proficiency, scored with 20 points (= 100%). First, laboratories had to detect the six CHIKV-reactive samples correctly for both IgM (if tested) and IgG antibodies. Second, neither antisera containing cross-reactive antibodies to heterologous arboviruses nor negative samples should give a positive result. Equivocal or borderline results were treated as positive. False-positive or false-negative results were not scored. Differentiation between IgM and/or IgG results was considered separately, and gave additional information concerning diagnostic quality. Collected data were analysed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). Results were analysed using the chi-square test. Whether or not common technical factors influenced the performance of participating laboratories was assessed by logistic regression.

Only six of 30 participating laboratories achieved all criteria for successful performance (Table 1). Thirteen laboratories gave at least 85% of accurate results. Lack of sensitivity for detection of IgM was observed for most laboratories scoring below 18 points. For performance under 70%, even the hypothesis of random expectation may not be ruled out. Interestingly, laboratories using the same commercial assay (Anti-Chikungunya virus IIFT IgM/IgG; Euroimmun AG) varied strongly in diagnostic accuracy. As commercial assays are produced according to certified criteria ensuring increased reproducibility of the results [9], false storage or improper handling of samples and assays is the most likely reason for this variability. Only three laboratories (nos. 5, 31 and 37) reported false-positive results on samples reactive for heterologous arboviruses or seronegative samples. Overall, the observed specificity was better than in past EQA studies [10–12]. As dengue virus and CHIKV co-circulate in some regions, causing similar clinical symptoms, differential diagnosis between these arboviral infections is very important.

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TABLE 1. Overall results of the external quality assurance for the serological detection of chikungunya virus (CHIKV) infection

		Sample no.											
		12	11	2	4	10	5	9	8	3	1		
Laboratory no.	Assay used	Anti-CHIKV +/+ (1 : 5)	Anti-CHIKV +/+ (1 : 10)	Anti-CHIKV +/+ (1 : 20)	Anti-CHIKV +/+ (1 : 40)	Anti-CHIKV +/+	Anti-CHIKV +/+	Anti-WNV –/–	Anti-DENV –/–	Negative –/–	Negative –/–	Score IgM/IgG	Correct results (%)
18[a]	EIA_{ih}/NT_{ih}	+/+	+/+	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/–	20	100
26	EIA_{ih}	+/+	+/+	+/+	+/+	+/+	+/+	–/–	–/–	–/–	–/–	20	100
12[a]	EIA_{ih}/**IFA$_{EI}$**	+/+	+/+	+/+	e/+	+/+	+/+	–/–	–/–	–/–	–/–	20	100
32	**IFA$_{EI}$**	+/+	+/+	+/+	+/+	(–)/+	+/+	–/–	–/–	–/–	–/–	19	95
20	**IFA$_{EI}$**	+/+	+/+	+/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	19	95
13	IFA_{ih}	+/+	+/+	e/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	19	95
39	**IFA$_{EI}$**	+/+	+/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	18	90
7	**IFA$_{EI}$**	+/+	(–)/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
11	**IFA$_{EI}$**	+/+	(–)/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
16	**IFA$_{EI}$**	+/+	(–)/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
23	**IFA$_{EI}$**	+/+	(–)/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
29	IFA_{ih}	+/+	(–)/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
30	**IFA$_{EI}$**	+/+	(–)/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
25	**IFA$_{EI}$**	+/+	+/+	(–)/+	(–)/(–)	+/+	+/+	–/–	–/–	–/–	–/–	17	85
1	IFA_{ih}	(–)/+	+/+	(–)/+	(–)/+	+/+	+/+	–/–	–/–	–/–	–/–	17	85
28[a]	EIA_{ih}/HIA_{ih}	+/+	(–)/+	(–)/+	(–)/+	(–)/+	(–)/+	–/–	–/–	–/–	–/–	15	75
4	IFA_{ih}	(–)/+	(–)/+	(–)/+	+/+	(–)/e	(–)/+	–/–	–/–	–/–	–/–	15	75
34	**IFA$_{EI}$**	(–)/+	(–)/+	(–)/+	(–)/+	+/+	(–)/+	–/–	–/–	–/–	–/–	15	75
36[a]	EIA_{CTK}/DB_{ih}	+/+	+/+	+/(–)	(–)/(–)	+/+	(–)/(–)	–/–	–/–	–/–	–/–	15	75
10	**IFA$_{EI}$**	(–)/+	(–)/+	(–)/+	(–)/+	(–)/+	(–)/+	–/–	–/–	–/–	–/–	14	70
22	EIA_{ih}	(–)/+	(–)/+	(–)/+	(–)/(–)	(–)/+	(–)/+	–/–	–/–	–/–	–/–	13	65
3	IFA_{ih}	(–)/+	(–)/+	(–)/(–)	(–)/+	(–)/+	(–)/+	–/–	–/–	–/–	–/–	13	65
5	EIA_{ih}	(–)/+	(–)/+	(–)/+	(–)/(–)	(–)/+	+/+	(+)/–	(+)/–	–/–	–/–	12	60
14	HIA_{ih}	+	+	+	+	+	+	–	–	–	–		100
15	HIA_{ih}	+	+	+	+	+	+	–	–	–	–		100
37	HIA_{ih}	+	+	+	+	+	+	e	–	e	e		70
9	IFA_{ih}[b]	ND/+	ND/+	ND/+	ND/+	ND/+	ND/+	ND/–	ND/–	ND/–	ND/–		100
6	IFA_{ih}[b]	ND/+	ND/+	ND/+	ND/(–)	ND/+	ND/+	ND/–	ND/–	ND/–	ND/–		90
31	EIA_{ih}[b]	ND/+	ND/+	ND/(–)	ND/(–)	ND/+	ND/e	ND/–	ND/–	ND/e	ND/–		70
27	EIA_{ih}[b]	ND/e	ND/(–)	ND/(–)	ND/(–)	ND/(–)	ND/+	ND/–	ND/–	ND/–	ND/–		60

Equivocal results were all treated as positive. However, those kind of results should be verified by a further approach. Assays marked with the index 'ih' are in-house assays. Assays marked in bold are commercial assays.
WNV, West Nile virus; DENV, dengue virus; x/x, IgM/IgG result; –, correct negative result; (–), false-negative result; +, correct positive result; (+), false-positive result; e, equivocal; ND, not done; DB, dot blot; EIA, enzyme immunoassay; HIA, haemagglutination inhibition assay; IFA, immunofluorescence assay; NT, neutralization test. Manufacturers: CTK, CTK Biotech Inc., San Diego, CA, USA; EI, Euroimmun AG, Lubeck, Germany.
[a]Laboratory reported combined results for both indicated test methods.
[b]Laboratory has not performed IgM testing.

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TABLE 2. Evaluation of the anti-chikungunya virus (CHIKV)-positive samples in the test panel[a]

Sample no.	Expected result, IgM/IgG	Fraction of correct classified results for IgM (%)	Fraction of correct classified results for IgG (%)
12	+/+	69.6 (16)	100.0 (23)
11	+/+	43.5 (10)	100.0 (23)
2	+/+	30.4 (7)	91.3 (21)
4	+/+	21.7 (5)	82.6 (19)
10	+/+	69.6 (16)	100.0 (23)
5	+/+	69.6 (16)	95.7 (22)
Overall	+/+	50.7[b]	94.9

[a]No. of laboratories, 23. Number of laboratories with correct results shown in parentheses.
[b]IgM antibodies are detected less frequently than IgG antibodies ($\chi^2 = 68.1$; $p<0.001$).

However, it should be noted that co-infections can be detected in some patients [13]. Table 2 shows that the sensitivity of IgM detection was significantly lower than that of IgG detection ($p < 0.001$). These results clearly demonstrate that either the assays have to be improved or that they were not performed correctly. As described in previous EQA studies [10–12], reliable assays for IgM detection are a prerequisite for correct diagnosis of acute or recent infections, and their development is therefore crucial. Thirteen laboratories used commercial tests (12 used Anti-Chikungunya virus IIFT IgM/IgG, Euroimmun AG; one used Chikungunya IgM Rapid Test, CTK Biotech Inc.) and 19 laboratories used in-house tests. In agreement with previous studies [10–12], no significant variation was found when comparing either assay type/format (enzyme immunoassay vs. immunofluorescence assay vs. haemagglutination inhibition assay) or assay origin (in-house assay vs. commercial assay) (data not shown).

This is the first multicentre EQA study of CHIKV serology to evaluate laboratory proficiency, indicating a need for certain laboratories to improve their test practice, in particular the detection of IgM antibodies. The great variation in the performance of different laboratories is similar to that observed in previous EQA studies [10–12]. Several laboratories recently and rapidly implemented CHIKV diagnostic assays, which may explain the great variability. However, for the clinical management of travellers returning from endemic areas to their non-endemic homelands, where pre-existing antibodies for alphaviruses are unlikely, the detection of IgG is considered to be a valid indicator of CHIKV infection [14]. For detecting acute cases, IgM provides only limited additional sensitivity. The lack of sensitivity in IgM detection may be compensated by simultaneous PCR testing. Comparative testing of well-characterized samples provided all participants with the opportunity to identify weaknesses and to improve methodologies, which should be confirmed in subsequent studies. New improved assays should detect the avidity of IgG to discriminate between acute primary and past infections in endemic countries, as IgM can persist for more than 1 year [9].

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Laboratories Participating in the EQA Study

The following 30 laboratories participated in the EQA study. Europe/Middle East: Medizinische Universität Wien, Vienna, Austria; Institute of Tropical Medicine, Antwerpen, Belgium; National Reference/WHO Collaborative Centre for Arboviruses & Haemorrhagic Fevers, Institut Pasteur, Lyon, France; Unite des Virus Emergents, Hopital La Timone, Marseille, France; CIBU Institut Pasteur, Paris, France; Bernhard Nocht Institut, Hamburg, Germany; EUROIMMUN AG, Lübeck, Germany; National Virus Reference Laboratory, University College Dublin, Dublin, Ireland; St Orsola University Hospital of Bologna, Bologna, Italy; Istituto Nazionale per le Malattie Infettive, Rome, Italy; Istituto Superiore di Sanità, Rome, Italy; Norwegian Institute of Public Health, Oslo, Norway; Instituto Nacional de Saúde Dr Ricardo Jorge (INSA), Lisboa, Portugal; University of Ljubljana, Ljubljana, Slovenia; National Centre for Microbiology, Majadahonda (Madrid), Spain; Swedish Institute for Infectious Disease Control, Solna, Sweden; University Hospitals of Geneva, Geneva, Switzerland; Spiez Laboratory, Spiez, Switzerland; Rijksinstituut voor Volksgezondheid (RIVM), Bilthoven, The Netherlands; Erasmus University Rotterdam, Rotterdam, The Netherlands; Pasteur Institute of Iran, Teheran, Iran; National Center for Zoonotic Viruses, Cahim Sheba Medical Centre, Tel Hashomer, Israel; Special Infectious Agents Unit, King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia. Asia: Public Health Laboratory Centre, Hong Kong; National Institute for Infectious Diseases, Tokyo, Japan; National Environmental Health Institute, Singapore. The Americas:

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The authors declare that they have no conflicts of interest in relation to this article.

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